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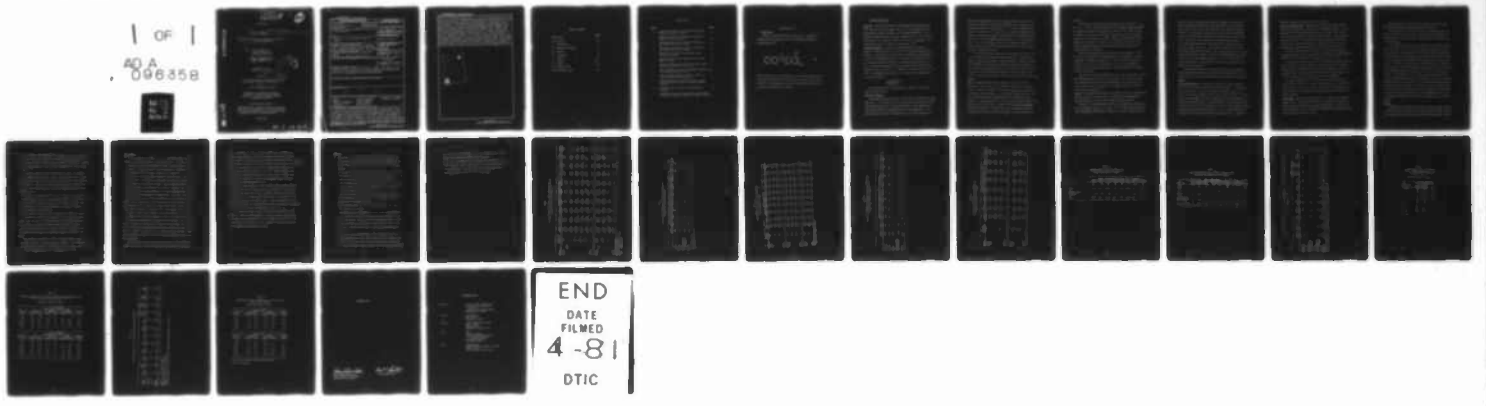

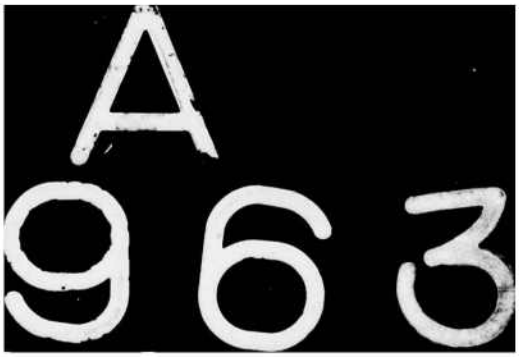
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Blood levels, excretion and tissue distribution of WR-158,122 ^{14}C were studied in 10 bile duct ligated rats, 10 bile duct cannulated rats, 8 control rats and in 2 control and one bile duct cannulated monkeys. In 72 hours bile duct ligated rats excreted about 16.3 ± 3.8% as ^{14}C in the urine and the remaining ^{14}C was recovered primarily in the feces. About 2% was recovered in the carcass and tissues at necropsy. Bile duct cannulated rats excreted about 10.9 ± 6.1% in the bile, 2.3 ± 1.3% in the urine and the remainder in the feces Only about 1.3 ± 1.0% was recovered in the carcass and tissues. Control rats

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excreted the compound rather rapidly; 1.5 ± 0.8% of the ^{14}C was recovered in the urine, 0.3 ± 0.2 in the carcass and other tissues and the remainder in the feces Total average recovery (as ^{14}C) in the three groups of rats varied from 94 to 100% of the dose. Two experiments were conducted in a single bile duct cannulated monkey. In the first the blood and plasma levels were highest at two hours after dosing. Plasma levels routinely exceeded levels in whole blood indicating little sequestration of WR-158,122 (as ^{14}C) in the red cells. Both levels were low at 24 hours and below detection at 48 hours. In this bile duct cannulated monkey biliary excretion over a 96 h. period accounted for 0.6 to 0.9% of the dose (as ^{14}C), urinary excretion for 3.1% and 87 to 95% of the dose was recovered in the feces. In two control monkeys urinary excretion over a 96 hour period amounted to 14 and 18%; 70% of the dose (as ^{14}C) was recovered in the feces. Excretion was essentially complete after 96 hours. In both rats and monkeys WR-158,122 appears to be rapidly but incompletely absorbed. Rats excreted 10% more in the bile but only 2-3% in the urine except in the case of bile duct ligated rats which excreted about 16% of the dose (as ^{14}C) in the urine.

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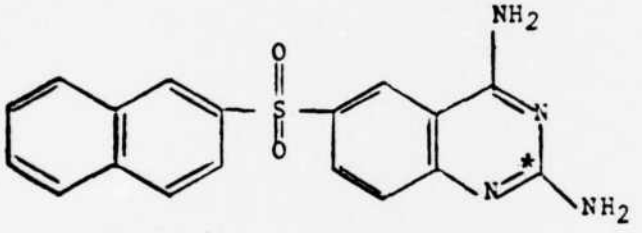
Interim Report No. 5

Introduction

Studies on the metabolic fate of WR-158,122 [2, 4-diamino-6-(2 naphthylsulfonyl)-quinazoline-2-^{14}C], the antifolate antimalarial compound shown below



$*^{14}C$

have been carried out in 3 rhesus monkeys, 10 bile-duct cannulated rats, 10 bile-duct ligated rats and 8 controls rats. Data on excretion and residual tissue distribution in rats and blood level data and excretion in monkeys are detailed in this report.

MATERIALS AND METHODS

Compounds. WR-158,122 [2,4-diamino-6-(2-naphthylsulfonyl)-quinazoline-2-^{14}C], empirical formula $C_{18}H_{14}SO_2N_4$, mol. wt. 350, was supplied by Walter Reed unlabeled as Bottle AY 65859. The ^{14}C-labeled preparation was synthesized by Research Triangle Institute and dated 1/8/79. Lot No. 2572-110, dated 8/20/79, has a specific activity of 69 µCi/mg or 24 mCi/mmole. The radiochemical purity in a number of TLC systems was > 98%.

Treatment Suspension. A typical treatment suspension was prepared as follows: 307.13 mg cold drug and 3.08 mg of ^{14}C labeled drug were ground intimately in a glass mortar with glass pestle with addition of small amounts of diluent (0.2% methylcellulose and 0.4% Tween 80 in distilled water) until a smooth suspension was achieved. The suspension was decanted into a tared round-bottom polycarbonate 200 ml centrifuge bottle containing glass beads and diluted to 124.08 ml. It contained 2.50 mg WR-158,122/ml and 1.86 µCi/ml. The suspension was stored at 4°C. Assay of this suspension gave the following results:

4,136,000 dpm/ml
1654 dpm/µg
1.86 µCi/ml

Other preparations with approximately the same activity were prepared in the same manner.

Analytical Procedures.

Blood samples were analyzed for total ^{14}C content as described in Interim Report No. 2. Urine samples from rats or monkeys were counted in Biofluor® (New England Nuclear Corp.). Feces were assayed in a few instances by the total sample digestion procedure described in Interim Report No. 1. Because we wished to identify fecal metabolites as well as measure total ^{14}C

content, most samples were milled in a stainless steel jar with stainless steel balls according to the procedure developed in this laboratory and described in detail in Interim Report No. 4. In brief, the samples were ground in the ball mill for 30 min. with 4 to 8 parts of anhydrous Na_2SO_4. Aliquots of the resulting fine off-white powder (250 mg) were digested for 48 hrs. with 1 ml of Soluene® (Packard Instrument Co.) and after bleaching with 0.5 ml of t-butyl hydrogen peroxide were counted in Biofluor. All samples were initially cooled and dark adapted and were internally standardized to determine efficiency.

The carcasses and other tissues (either separately in some cases or as a unit in others) were counted as described previously. The bones do not dissolve in the alkaline digestion procedure employed. However, digestion with Soluene® of the dried finely powdered bones from some representative rats yielded counts equivalent only to 0.01% of the dose. Extraction of the bone powder with tetrahydrofuran recovered even smaller amounts of drug (as ^{14}C). Based on these findings no further work on bone ^{14}C has been done.

Animals.

Rats. Sprague-Dawley-derived rats weighed 244 to 305 g (bile duct ligated), 260 to 400 g (bile duct cannulated) and 365 to 410 g (control rats) at the time of treatment. The bile duct ligated rats were prepared as described in Interim Report No. 1 and housed in metabolism cages. The bile duct cannulated rats, prepared essentially as described in Interim Report No. 1, were restrained in a close fitting wire housing attached to the metal cover of a plastic cage. Food and water were supplied ad libitum four hours after dosing. Urine and feces were separated using a bottle equipped with a funnel and woven wire separator. Bile was collected in a

tared vial.

Bile, urine and feces were collected usually at 12, 24, and 48 h. At 48 h. or 72 h. the rats were necropsied using an anesthetic dose of Nembutal®. In a number of instances the tissues were assayed for ^{14}C in three sections: 1) tissues (liver, heart, lungs, spleen and kidneys as a pool); 2) gastrointestinal tract and contents; 3) carcass. However, these analyses have been reported as a single item, "carcass with all tissues", since with rare and usually unexplained exceptions these three tissue groups accounted for no more than about 2% of the dose.

Ten bile duct ligated rats were prepared and all are reported. Of the 20 bile duct cannulated that were prepared only 10 are reported. Four died prematurely; 3 exhibited unacceptably low recovery; two had large amounts of drug in the GI tract not characteristic of any of the other animals in the group; and one was eliminated because it had unaccountably large amounts of ^{14}C in the carcass.

Twelve control rats were given WR-158,122 but only 8 are reported. Three were deleted because of low recovery (50 to 60%) of the ^{14}C dose. The basis for these poor recoveries has not been established. One was eliminated because about half the dose was present in the carcass whereas the other carcass values were well below 1%.

Monkeys. Two unoperated rhesus monkeys (# 1449♀ and # 709♀) were administered WR-158,122 while in a primate chair. Both animals required supplemental feeding, possibly due to the stress associated with the restraint.

The bile duct cannulated monkey (75-5♀) was acclimated to a primate chair for considerable periods prior to surgery. The cannulation was accomplished under Ketamine®-Surital® anesthesia following a 24 h fast. Blood chemistries

and CBC were done on a blood sample obtained at the time of the operation. After the operation the animal was given Flocillin® (1 ml BID) and intravenous fluids. When no bile flow occurred on day 4 surgery was repeated. Following a two week period during which the animal required considerable support in the form of bile, fluids and liquid diet given via nasal catheter or duodenal tube, the animal's bile flow became more consistent. On the twentieth day following the second operation, the animal was given a single oral dose of WR-158,122. During the post treatment period saline was slowly infused via the duodenal tube and the animal's bile was replaced with about equal amounts of beef bile. Blood samples were obtained from the left or right antecubital veins at 2, 4, 6, 8, 12, 24, 48 and 72 hours after treatment. Bile production and feces and urine excretion were excellent during this period.

A second dose of WR-158,122 was administered at 4 days. During the second post-operative period the animal also required supportive fluids and beef bile but bile, urine and feces production was good during this period.

Results

Bile Duct Ligated Rats. The excretion of single oral doses of labelled WR-158,122 in 10 bile duct ligated rats is shown in Table 1. Urinary excretion varied from 11 to 21% and was essentially complete at the end of 48 hours. Fecal excretion varied from 74 to 93 per cent of the dose. The high value for total recovery in BL-10 is probably due to an undetected error in measuring the treatment dose. As one would expect, the 4 rats sacrificed at 48 hours (Bl-15 to Bl-18) had more drug (1 to 11%) in their tissues at necropsy than the remaining animals killed after 72 hr (0.3 to 0.7%) (see Table 2), but most of the drug was cleared from the rat's body by 72 hr. Additional

comments on these data will occur later in the report.

Bile Duct Cannulated Rats. The excretion of single oral doses of labeled WR-158,122 in 10 bile duct cannulated rats is described in Table 3. Biliary excretion (as ^{14}C) varied rather widely from 2.5 to 23.6%. This variation may reflect the adequacy of biliary excretory processes but does not seem to depend on bile flow. By far the most active bile excretion occurred during the first 12 hours after treatment; the fraction of the total biliary excretion which occurred during this first period varied from 59 to 87%.

Urinary excretion of WR-158,122 (as ^{14}C) in bile duct cannulated rats accounted for from 0.8 to 4.2% of the dose, the majority of the radiolabel being excreted via this route usually appearing during the first 12 hours.

Fecal excretion of WR-158,122 (as ^{14}C) in bile duct cannulated rats varied from 64 to 94% of the dose. Excretion via this route occurred principally during the first 24 hr period and was essentially complete by 48 hours, as indicated by the finding that less than 1% of the dose was recovered in the GI tract and contents at 48 hours when the rats were necropsied.

The data on recovery of ^{14}C in the carcass and tissues presented in Table 4 show that from 0.42 to 3.45% of the dose was still in the body at the end of 48 hours. As a rule the amount of drug (as ^{14}C) in the body at this time was 1% or less, clearance of drug from the body being essentially complete by 48 hours.

Control Rats. The parallel results obtained in 8 control rats are shown in Tables 5 and 6. The kinetics of excretion were essentially the same as observed in bile duct ligated and bile duct cannulated rats. Urinary excretion accounted for 0.8 to 2.9% of the ^{14}C administered and occurred primarily (68 to 91%) during the first 12 hours.

Fecal excretion (as ^{14}C) accounted for from 91 to 109% of the dose, the principal portion occurring during the first 24 hours.

The drug content (as ^{14}C) of the carcass at 48 hours never accounted for more than about 0.5% of the dose. This finding further supports the suggestion that in rats WR-158,122 is rapidly cleared from the body after single oral doses.

If we compare data in bile duct ligated, bile duct cannulated and control rats (Table 7) one is led to some interesting conclusions. Comparison of the data on urinary excretion of WR-158,122 in bile cannulated rats with the data in control (unoperated) rats suggests that about equal amounts of the drug undergo urinary excretion whether the bile duct is cannulated or not. If the duct is ligated, however, considerably more ^{14}C appears in the urine (16%). These findings suggest that at least 13 to 16% of the dose must be absorbed, some being excreted in the urine (1.6-2.3%), the rest in the bile. The assumption that the livers of these bile duct ligated and bile duct cannulated animals may not be functioning as well as in unoperated animals suggests that perhaps even more of the dose normally undergoes absorption and biliary excretion. The fact that the drug is cleared rapidly from the GI tract (data obtained but not shown) suggests that that portion of the drug undergoing biliary excretion is not reabsorbed. Perhaps the only metabolites excreted in the bile are conjugates too polar to be reabsorbed.

Monkeys

Although biliary cannulation was carried out in four monkeys, only one (75-5♀) survived for sufficient time to permit metabolism studies. No pertinent reasons have been discovered to account for this rather poor survival

record. In Table 8 blood chemistries on 75-5♀ at the time of initial surgery and at two periods thereafter are compared with normal values. The animal had high levels of LDH and alkaline phosphatase and slightly elevated SGOT even before surgery; values for other parameters were close to normal values.

Following a single oral dose of WR-158,122 (5 mg/kg), the highest blood or plasma level occurred at 2 hours; then the levels fell to almost insignificant values by 24 hours and were below the level of detection thereafter (Table 9). Because of the stress associated with collecting blood samples in this monkey, blood levels were not determined during the second treatment period.

Excretion of WR-158,122 (as ^{14}C) in bile, urine and feces following two single oral doses of WR-158,122 are detailed in Table 10. Recovery of drug (as ^{14}C) in bile was quite low, equal to less than 1% of the dose. About 3% was recovered in the urine and the remainder in the feces. Comments on these data appear in the discussion.

Two unoperated monkeys were used to evaluate the absorption and excretion of WR-158,122. The clinical chemistry findings (SMA 12/60) compared with results in a group of 20 healthy female rhesus monkeys studied by the same laboratory but housed in another building are shown in Table 11. Except for the elevated LDH values for which we have no explanation, these two female animals appeared to be relatively normal, as judged by these data.

The excretion of WR-158,122 (as ^{14}C) in these two female animals following single oral doses of 5 mg/kg are shown in Table 12. It is interesting that although total recovery of ^{14}C amounted to only 84 to 87%, from 14 to 18% of the ^{14}C appeared in the urine, the remainder in the feces.

Discussion

It is interesting to compare the data in this Interim Report with the results we obtained previously in Interim Report No. 1. In rats with bile duct ligation, the greatest difference appeared in fecal recovery (81.5% in current group versus 67.8% in the Interim Report No. 1 group) and higher total recovery (99.9% in Interim 5 versus 88.4% in Interim 1).

Excretion in bile was slightly greater in the early group of bile-duct cannulated rats. In the bile duct cannulated rats there was a greater disparity between the two groups of animals. Thus we recovered almost 30% of the dose of WR-158,122 in the bile in the Interim 1 group but only 11% in the current series. The simplest explanation for this discrepancy might be that the bile duct cannulae appeared to function better in the first group of rats. Comparison of the kinetics of biliary excretion in the two groups indicates another striking difference. In the first group of six rats (Interim 1) the peak excretion of ^{14}C in the bile occurred in the 12-24 hr. sample in 3 rats, in the 24-48 hr sample in 2 rats and in the 0-12 hr sample in only 1 rat. In contrast, in the 10 rats included in this interim report all of the rats gave maximum values for biliary excretion in the 0-12 hr period after which the excretion rates dropped rather rapidly in most instances. The basis for this difference has not been ascertained but one would reason that if the biliary cannulae are functioning well excretion via this route would be greater than in a group of rats with less well functioning biliary cannulae. Perhaps we need additional criteria on which to evaluate the adequacy of the cannulation procedure.

The monkey data suffer from their paucity as well as from the general comment that the data in this one bile duct cannulated monkey are probably

not very representative of data one should expect in healthy monkeys with adequate biliary cannulae. Thus one would expect that the total excretion of WR-158,122 (^{14}C) in bile and urine in monkeys with well functioning bile duct cannulae should at least equal the urinary excretion of the drug in intact monkeys. If the drug is excreted in the bile as a non-reabsorbable metabolite then combined urine and biliary excretion in bile duct cannulated animals could very well exceed the urinary levels observed in intact animals. However, such has not been our experience to date and this suggests that the biliary cannulation technique needs improvement.

It might be worth commenting in this connection that urinary excretion of ^{14}C in control rats given single oral doses of WR-158,122-^{14}C was about one order of magnitude less than the corresponding figures in either bile duct ligated rats or bile duct cannulated rats (when in the latter one adds the biliary excretion to the urinary values). The best explanation of this would appear to be that the rat excretes WR-158,122 as metabolites (conjugates) in the bile that are unable to be reabsorbed from the gut.

In the unoperated monkey, urinary excretion levels are about 10 times as high as in the rat and this suggests either that the total metabolites excreted in the bile are considerably less in the monkey than in the rat or that the form in which the monkey excretes the compound in the bile is much more readily reabsorbed and excreted in the urine.

Summary

1. Blood levels, excretion and tissue distribution of WR-158,122 ^{14}C were studied in 10 bile duct ligated rats, 10 bile duct cannulated rats, 8 control rats and in 2 control and one bile duct cannulated monkeys.
2. In 72 hours bile duct ligated rats excreted about 16.3 ± 3.8% as ^{14}C in the urine and the remaining ^{14}C was recovered primarily in the feces. About 2% was recovered in the carcass and tissues at necropsy.
3. Bile duct cannulated rats excreted about 10.9 ± 6.1% in the bile, 2.3 ± 1.3% in the urine and the remainder in the feces. Only about 1.3 ± 1.0% was recovered in the carcass and tissues.
4. Control rats excreted the compound rather rapidly; 1.5 ± 0.8% of the ^{14}C was recovered in the urine, 0.3 ± 0.2 in the carcass and other tissues and the remainder in the feces.
5. Total average recovery (as ^{14}C) in the three groups of rats varied from 94 to 100% of the dose.
6. Two experiments were conducted in a single bile duct cannulated monkey. In the first the blood and plasma levels were highest at two hours after dosing. Plasma levels routinely exceeded levels in whole blood indicating little sequestration of WR-158,122 (as ^{14}C) in the red cells. Both levels were low at 24 hours and below detection at 48 hours.
7. In this bile duct cannulated monkey biliary excretion over a 96 h. period accounted for 0.6 to 0.9% of the dose (as ^{14}C), urinary excretion for 3.1% and 87 to 95% of the dose was recovered in the feces.
8. In two control monkeys urinary excretion over a 96 hour period amounted

to 14 and 18%; 70% of the dose (as ^{14}C) was recovered in the feces. Excretion was essentially complete after 96 hours.

9. In both rats and monkeys WR-158,122 appears to be rapidly but incompletely absorbed. Rats excreted 10% more in the bile but only 2-3% in the urine except in the case of bile duct ligated rats which excreted about 16% of the dose (as ^{14}C) in the urine.

Table 1

Excretion of WR-158,122 in Bile Duct Ligated Rats
Single Oral Dose of 10 mg/kg

Sample	Hours Post Dose	Percent Dose Recovered as ^{14}C									
		BL-9	BL-10	BL-11	BL-12	BL-13	BL-14	BL-15	BL-16	BL-17	BL-18
Urine (ml)	6	1.4 (10.0)	1.5 (7.2)	1.1 (10.0)	1.2 (9.9)	3.1 (11.3)	1.0 (10.2)	*NC	NC	NC	NC
	12	6.1 (10.3)	5.5 (13.0)	7.4 (11.2)	4.9 (10.7)	6.0 (9.9)	5.1 (6.7)	5.8 (8.5)	6.5 (16.5)	6.8 (14.0)	6.6 (13.0)
	24	3.9 (10.8)	6.2 (12.4)	10.1 (10.2)	6.1 (10.4)	7.0 (10.7)	1.5 (10.9)	5.3 (20.5)	2.4 (31.5)	5.6 (34.0)	4.8 (14.0)
	48	1.6 (27.5)	6.7 (41.0)	2.0 (13.5)	4.4 (23.0)	4.4 (51.0)	5.1 (33.5)	1.6 (35.5)	2.2 (35.5)	5.7 (30.0)	3.9 (30.5)
	72	0.23 (15.0)	0.83 (18.5)	0.42 (16.5)	0.59 (15.0)	0.45 (10.5)	0.41 (15.0)	**SAC	SAC	SAC	SAC
Total		13.2	20.7	21.0	17.2	21.0	13.1	12.7	11.1	18.1	15.3
Feces (g)	24	79.1 (3.0)	82.3 (3.8)	69.3 (5.1)	69.8 (3.3)	71.2 (4.9)	65.5 (4.7)	63.5 (3.3)	84.3 (8.0)	68.4 (6.6)	60.7 (1.9)
	48	5.2 (4.2)	10.8 (5.9)	4.6 (5.5)	11.0 (2.6)	6.4 (4.0)	14.5 (3.7)	14.4 (2.8)	2.1 (3.5)	12.0 (4.3)	17.2 (1.6)
	72	0.39 (4.8)	0.23 (4.4)	0.10 (3.6)	1.5 (5.1)	S.L.+	1.0 (2.7)	SAC	SAC	SAC	SAC
Total		84.7	93.3	74.0	82.3	77.6	81.0	77.9	86.4	80.4	77.9

*NC = No collection

**SAC = Sacrificed at 48 hrs.

+S.L. = Sample Lost

Table 2

Recovery of WR-158,122 in Bile Duct Ligated Rats
Single Oral Dose of 10 mg/kg

	Percent of Dose Recovered (as ^{14}C) in 48 or 72 Hours									
	BL-9	BL-10	BL-11	BL-12	BL-13	BL-14	*BL-15	*BL-16	*BL-17	*BL-18
Urine	13.2	20.7	21.0	17.2	21.0	13.1	12.7	11.1	18.1	15.3
Feces	84.7	93.3	74.0	82.3	77.6	81.0	77.9	86.4	80.4	77.9
Carcass with all tissues	0.36	0.53	0.35	0.53	0.46	0.72	0.93	2.4	2.6	10.9
Total	98.3	114.5	95.4	100.0	99.1	94.8	91.5	99.9	101.1	104.1

*Sacrificed at 48 hours.

Table 3

Excretion of WR-158, 122 in Bile Duct Cannulated Rats

Single Oral Dose of 10 mg/kg

Sample	Hours Post Dose	Percent Dose Recovered as ^{14}C									
		BC-13	BC-14	BC-15	BC-17	BC-18	BC-21	BC-22	BC-23	BC-24	BC-26
Bile	12	16.3	7.4	8.6	3.4	2.1	9.1	10.4	10.1	4.7	6.3
(g)		(8.3)	(6.7)	(6.8)	(8.3)	(8.2)	(10.1)	(8.8)	(7.8)	(7.4)	(7.7)
	24	5.2	1.7	4.7	0.37	0.27	0.95	4.4	1.4	2.2	0.91
		(8.0)	(13.5)	(6.4)	(3.7)	(7.3)	(9.1)	(8.9)	(7.0)	(10.2)	(8.3)
	48	2.1	0.52	1.9	0.32	0.14	0.40	0.68	0.60	1.1	0.48
		(17.9)	(16.4)	(13.6)	(14.8)	(16.8)	(21.0)*	(21.2)*	(47.0)*	(27.9)*	(15.2)
Total		23.6	9.6	15.2	4.1	2.5	10.5	15.5	12.1	8.0	7.7
Urine	12	1.5	1.3	1.1	0.60	0.39	3.33	1.5	1.5	0.93	1.0
(ml)		(9.4)	(2.9)	(3.4)	(6.6)	(10.6)	(13.2)	(8.8)	(10.8)	(15.8)	(4.7)
	24	0.68	0.57	2.1	0.13	0.08	0.35	1.9	0.34	0.39	0.16
		(13.0)	(15.6)	(8.3)	(6.3)	(12.6)	(6.3)	(10.2)	(9.0)	(11.8)	(4.7)
	48	0.31	0.20	1.0	0.07	0.15	0.19	0.33	0.28	0.32	0.13
		(26.3)	(17.6)	(26.4)	(12.6)	(19.6)	(57.0)	(54.0)	(41.0)	(25.2)	(17.8)
Total		2.5	2.1	4.2	0.80	0.62	3.9	3.7	2.1	1.6	1.3
Feces	24	54.4	62.4	82.4	63.2	82.4	88.5	78.8	68.3	81.3	71.9
(g)		(3.0)	(6.5)	(2.2)	(3.1)	(8.8)	(17.6)	(6.6)	(4.3)	(7.3)	(2.1)
	48	10.0	4.8	12.0	15.6	1.4	1.7	3.6	7.1	3.9	20.4
		(10.5)	(14.3)	(9.0)	(6.1)	(5.2)	(22.7)	(8.5)	(6.4)	(7.2)	(7.9)
TOTAL		64.4	67.2	94.4	78.8	83.8	90.2	82.4	75.4	85.2	92.3

* Sample volume increased with water

Table 4

Recovery of WR-158,122 from Bile Duct Cannulated Rats
Single Oral Dose of 10 mg/kg

	Percent of Dose Recovered (as ^{14}C) in 48 Hours									
	BC-13	BC-14	BC-15	BC-17	BC-18	BC-21	BC-22	BC-23	BC-24	BC-26
Urine	2.5	2.1	4.2	0.80	0.62	3.9	3.7	2.1	1.6	1.3
Feces	64.4	67.2	94.4	78.8	83.8	90.2	82.4	75.4	85.2	92.3
Bile	23.6	9.6	15.2	4.1	2.5	10.5	15.5	12.1	8.0	7.7
Carcass with all tissues	1.37	0.56	3.45	1.03	2.35	0.76	0.42	0.71	1.94	0.66
Total	91.9	79.5	117.3	84.7	89.3	105.4	102.0	90.3	96.7	102.0

Table 5

Excretion of WR-158,122 in Control Rats
Single Oral Dose of 10 mg/kg

Sample	Hours Post Dose	Percent Dose Recovered as ^{14}C							
		CT-1	CT-2	CT-3	CT-4	CT-8	CT-9	CT-11	CT-12
Urine (ml)	12	1.7 (13.0)	1.2 (4.7)	2.0 (11.0)	1.3 (8.0)	0.74 (8.8)	1.3 (8.8)	0.60 (7.5)	0.68 (6.1)
	24	0.73 (14.5)	0.12 (8.0)	0.8 (10.8)	0.28 (13.0)	0.06 ((5.5)	0.38 (13.9)	0.07 (11.8)	0.11 (10.3)
	48	0.07 (31.0)	0.03 (14.5)	0.13 (24.0)	0.04 (18.0)	0.01 (7.1)	0.03 (19.4)	0.02 (32.0)	0.03 (7.2)
Total		2.5	1.4	2.9	1.6	0.81	1.7	0.69	0.82
Feces (g)	24	83.0 (7.3)	95.9 (11.1)	97.5 (11.1)	90.8 (11.4)	84.5 (13.1)	107.3 (11.3)	94.3 (11.2)	90.4 (9.3)
	48	16.1 7.1	2.8 (10.5)	2.8 (8.1)	6.5 (15.3)	6.1 (21.6)	1.3 (13.6)	1.4 (4.3)	5.0 (8.4)
Total		99.1	98.7	100.3	97.3	90.6	108.6	95.7	95.4

Table 6

Recovery WR-158,122 from Control Rats
Single Oral Dose of 10 mg/kg

	Percent of Dose Recovered (as ^{14}C) in 48 Hours							
	CT-1	CT-2	CT-3	CT-4	CT-8	CT-9	CT-11	CT-12
Urine	2.5	1.4	2.9	1.6	0.81	1.7	0.69	0.82
Feces	99.1	98.7	100.3	97.3	90.6	108.6	95.7	95.4
Carcass with all tissues	0.66	0.23	0.45	0.21	0.12	0.13	0.37	0.28
Total	102.3	100.3	103.7	99.1	91.5	110.4	96.8	96.5

Table 7

Recovery of WR-158,122 (as ^{14}C) in Bile Duct Ligated, Bile-Duct Cannulated and Control Rats

	In Percent Dose								
	Bile-Duct Ligated			Bile-Duct Cannulated			Controls		
Sample	N	x	SD	N	x	SD	N	x	SD
Urine	10	16.34	3.78	10	2.28	1.28	8	1.55	0.81
Bile				10	10.88	6.14			
Feces	10	81.55	5.51	10	81.41	10.10	8	98.21	5.16
Carcass with all tissues	10	1.98	3.24	10	1.33	0.98	8	0.31	0.18
Total recovery	10	99.87	6.26	10	95.91	11.10	8	100.1	5.64

Table 8

SMA 12/60 Assays on a Bile Duct Cannulated Monkey Prior to Treatment With WR-158, 122 ^{14}C

(Rhesus 75-5 ♀)

	Sugar mg %	Uric Acid mg %	Chol mg %	SGOT mU/ml	LDH mU/ml	P mg %	Alk P mU/ml	Ca mg %	Direct Bili-rubin mg %	Total Bili-rubin mg %	Protein g %	Alb g %
Surgery 2-15-80	87	0.2	110	45	493	6.3	>350	10.8	0.2	0.5	8.1	4.0
2-25-80 7 days after 2nd surgery	79	0.7	80	40	355	4.5	>350	9.0	0.3	0.5	6.5	3.2
3-7-80 3 days before 1st treatment	85	0.3	80	40	330	5.9	>350	9.8	0.2	0.3	7.3	3.5
Normal* Values	89 ±3.5	0.61 ±0.05	170.2 ±6.0	30.4 ±2.2	271.2 ±24.6	3.56 ±0.21	166.3 ±17.5	9.78 ±0.16	0.12 ±0.01	0.26 ±0.02	8.01 ±0.13	3.77 ±0.11

* Based on assays of 20 rhesus female monkeys by same laboratory. (mean ± S.E.)

Table 9

Blood and Plasma Levels of WR-158,122 (as ^{14}C) in a Bile Duct Cannulated Monkey (75-5 ♀)

Single Oral Dose of 5 mg/kg

Hours Post Dose	First Treatment µg/g as ^{14}C		
	Blood	Plasma	Hct
2	0.33	0.52	41
4	0.26	0.40	40
6	0.17	N.S.*	N.S.*
8	0.13	0.20	40
12	0.05	0.09	40
24	0.02	0.05	42
49	<0.01	<0.01	38
72½	<0.01	<0.01	40

*No sample

Table 10

Cumulative Excretion of WR-158,122 (as ^{14}C) in Bile, Urine and Feces from a Bile Duct Cannulated Monkey (75-5 ♀)

Single Oral Doses of 5 mg/kg

	First Treatment Excretion in Percent of Dose						
Hours Post Dose	Bile		Urine		Feces		Combined
	Period	Total	Period	Total	Period	Total	Total
0-12	0.46	0.46	1.9	1.9			2.4
12-24	0.21	0.67	0.49	2.4	48.4	48.4	51.5
24-48	0.20	0.87	0.59	3.0	36.9	85.3	89.2
48-72	0.03	0.90	0.05	3.1	1.2	86.5	90.5
72-96	0.01	0.91	<0.01	3.1	0.10	86.6	90.6

	Second Treatment Excretion in Percent of Dose						
Hours Post Dose	Bile		Urine		Feces		Combined
	Period	Total	Period	Total	Period	Total	Total
0-12	0.26	0.26	1.5	1.5			1.8
12-24	0.21	0.47	1.1	2.6	35.8	35.8	38.9
24-48	0.13	0.60	0.37	3.0	57.9	93.7	97.3
48-72	0.01	0.61	0.11	3.1	1.0	94.7	98.4
72-96	0.01	0.62	0.02	3.1	0.09	94.8	98.5
96-120	<0.01	0.62	0.01	3.1	0.05	94.9	98.6

Table 11

SMA 12/60 Results on Two Control Monkeys Before Treatment

Monkey No.	Sugar mg %	Uric Acid mg %	Chol mg %	SGOT mU/ml	LDH mU/ml	P mg %	Alk. P mU/ml	Ca mg %	Direct Bili-rubin mg %	Total Bili-rubin mg %	Pro-tein g %	Alb g %
1449 ♀*	60	0.4	205	42	481	3.2	235	9.3	0.0	0.1	7.5	3.9
709 ♀**	51	0.3	170	44	446	2.5	151	9.6	0.0	0.1	7.3	3.9
Normal Values***	89 ±3.5	0.61 ±0.05	170.2 ±6.0	30.4 ±2.2	271.2 ±24.6	3.56 ±0.21	166.3 ±17.5	9.78 ±0.16	0.12 ±0.01	0.26 ±0.02	8.01 ±0.13	3.77 ±0.11

* assayed 2 days before treatment

** assayed 15 days before treatment

*** based on assays of 20 rhesus female monkeys by same laboratory (mean ± S.E.)

Table 12

Cumulative Excretion of WR-158,122 in Urine and Feces of Two Control Monkeys

Single Oral Doses of 5 mg/kg

Rhesus 1449 ♀					
Hours Post Dose	Urine*		Feces		Combined Total
	Period	Total	Period	Total	
0-12	2.6	2.6	0.02	0.02	2.6
12-24	7.8	10.4	0.17	0.19	10.6
24-48	2.9	13.3	51.9	52.1	65.4
48-72	0.59	13.9	11.0	63.1	77.0
72-96	0.15	14.1	6.9	70.0	84.1

Rhesus 709 ♀					
Hours Post Dose	Urine		Feces		Combined Total
	Period	Total	Period	Total	
0-12	6.0	6.0	N.S.**		6.0
12-24	8.5	14.5	0.13	0.13	14.6
24-48	2.2	16.7	0.02	0.15	16.9
48-72	0.71	17.4	58.3	58.5	75.9
72-96	0.24	17.6	10.5	69.0	86.6
96-120	0.05	17.7	0.59	69.6	87.3

* 24 and 48 hr. samples contaminated with menses

** N.S. = No Sample

SIGNATURE PAGE

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Acting Principal Investigator
1980-1981 Contract Period

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Sample	Hou
Urine (ml)	
Total	
Feces (g)	
Total	

*NC = No colle
**SAC = Sacrif
+S.L. = Sample

END
DATE
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Monkey No.	Sugar mg %
1449 ♀*	60
709 ♀**	51
Normal Values***	89 ±3.5

*assayed 2 days be

**assayed 15 days

***based on assay